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OBSERVATIONS ON THE FEEDING MECHANISM, DIET AND DIGESTIVE PHYSIOLOGY OF *HISTRIOBDELLA HOMARI* VAN BENEDEN 1858: AN ABERRANT POLYCHAETE SYMBIOTIC WITH NORTH AMERICAN AND EUROPEAN LOBSTERS

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Although the Polychaeta are predominantly free-living annelids, the class includes a number of symbiotic species which live in partnerships of various degrees of intimacy and dependency with host organisms from many different phyla (Clark, 1956; Fauvel, 1959). Relatively little is known of the precise nature of these associations; they are probably based on shelter and nutritional factors, but most of the polychaetes involved do not show the adaptive structural modifications which might be expected and which are evident in other symbiotic annelids such as the Myzostomaria and in symbiotic flatworms such as the Monogenea, Digenea and Cestoda. In these four taxa, however, symbiosis is the only life style; the structural modifications are accompanied, in the flatworms at least, by physiological adaptations which demonstrate the firm nutritional basis of the relationship with the host, and the symbiotes are clearly recognizable as specialized parasites. In contrast, the symbiotic life style is of only sporadic occurrence in the Polychaeta, and in this respect the symbiotic polychaetes are more comparable to those turbellarian flatworms which have adopted symbiotic habits. These too, when compared with their more abundant free-living relatives, show few changes in structure (Hyman, 1951) or, indeed, in diet, feeding mechanisms and digestive physiology (Jennings, 1971), but some species do show differences in food reserves and reproductive strategies which can be related to their life style (Jennings, 1973; Calow and Jennings, 1974). These species are among the most highly adapted of the symbiotic Turbellaria and illustrate possible stages in the evolution of the obligate entoparasites which constitute the wholly symbiotic classes of their phylum.

It is not known whether there is a comparable situation, as regards general nutritional physiology, in symbiotic polychaetes. Of these, the most highly modified morphologically are the members of the family Histriobdellidae Vaillant 1890, all of which live symbiotically in the branchial chamber or on the ventral body surface of crustacean hosts. The histriobdellids are small annelids, rarely exceeding 2 mm in length; they have only nine post-cephalic segments of which the last is bifurcated to form a pair of locomotor-cum-adhesive organs, and segmental appendages are much reduced or absent. Despite these modifications the Histriobdellidae show undoubted affinities with the Eunicida (Mesnil and Caullery, 1922; Remane, 1932; Hermans, 1969), and their position in that order has been confirmed from a study of their nervous system (Gelder and Jennings, 1975).

The family consists of the two genera *Histriobdella* and *Stratiodrilus,* the former with one species *H. homari* van Beneden 1858 symbiotic with marine

lobsters in northern European and northeastern American waters (van Beneden, 1853; 1858; Uzmann, 1967) and the latter with four species symbiotic with freshwater Decapoda in Tasmania, Australia, Uruguay, Madagascar, Chile, Argentina and Patagonia, and one with a marine isopod in South Africa (Haswell, 1900; 1913; Cordero, 1927; Harrison, 1928; Lang, 1950; Roubaud, 1962; Führ, 1971).

The unusual features of the Histriobdellidae suggest that they may represent one climax in the evolution of symbiotic habits within the Polychaeta, comparable perhaps to some turbellarian symbioses such as those involving temnocephalid rhabdocoels and decapod crustaceans or umagillid rhabdocoels and echinoids (Jennings, 1971). The nature of the histriobdellid-crustacean relationship, however, has not been defined; the histriobdellids are usually described as parasites (*e.g.*, Mesnil and Caullery, 1922; Fauvel, 1959; Dales, 1967), but no supporting evidence has been given. Virtually nothing is known of their diets or feeding mechanisms; the proboscis and jaws are much modified, but judging from the descriptions by Foettinger (1884), Shearer (1910), Haswell (1913), Mesnil and Caullery (1922) and Roubaud (1962), they are clearly derived from the basic eunicid pattern described by Fauvel (1959) and Dales (1962), suggesting a scraping or browsing type of feeding. The life history is simple, with the females attaching their eggs to the ventral surface and egg masses of the host and the young hatching as miniature adults (van Beneden, 1858; Haswell, 1913). Transference between hosts is probably accomplished by direct migration, as demonstrated by Simon (1967; 1968) for *H. homari* under laboratory conditions.

H. homari is a common symbiote of European and North American lobsters (van Beneden, 1858; Uzmann, 1967) and, if parasitic, may be of some economic importance. It shows all the unusual features of its family and it has, therefore, been selected for study as an example, albeit a somewhat extreme one, of symbiosis in the Polychaeta. Since most symbioses have a nutritional basis, the investigation has been concerned with the structure of the alimentary canal, the diet, feeding mechanism and general digestive physiology, with the aim of establishing the status of *H. homari vis-à-vis* its host and facilitating comparisons of polychaete symbioses with those found in the Platyhelminthes and other phyla.

Materials and Methods

Histriobdella homari was collected from the gills and epipodites of lobsters, *Homarus americanus,* caught at Nahant and Woods Hole, Massachusetts, and *H. vulgaris* caught at Whitby, England.

The structure of the buccal cavity, proboscis, alimentary canal and their associated glands, and the nature and role of the various substances secreted by the latter, were studied by histological and histochemical methods. For histological and nonenzymic histochemical studies, specimens were fixed in marine Bouin's fluid, Flemming's fixative, 10% neutral formalin or 95% ethanol. Whole mounts and serial sections cut at 5 μm after dehydration in graded ethanols and impregnation in polyester wax (melting point, 39° C) were stained with Ehrlich's haematoxylin and eosin, Gram's stain or Mallory's triple stain, or by the periodic acid-Schiff reaction for carbohydrates and acid mucopolysaccharides, Steedman's Alcian blue method for acid mucopolysaccharides, Feulgen's reaction for DNA, the

Sudan IV and Oil red 0 methods for lipids and Best's carmine method for glycogen.

For histochemical studies of digestive enzymes fixation was in 10% formalin, buffered with phosphate to pH 7.0, at 1° C. Whole mounts, and serial sections cut at 10 μm after dehydration in graded acetones and impregnation in paraffin wax (melting point, 45° C), were examined by the indoxyl acetate method for non-specific esterases (Holt, 1958), the L-leucyl β-naphthylamide hydrochloride method for arylamidases (Burstone and Folk, 1956), the naphthyl AS-BI phosphate methods for acid and alkaline phosphatases (Burstone, 1958) and the post-coupling 6 bromo-2-naphthyl-β-D-glucopyruronoside (glucuronide) method for β-glucuronidase (Pearse, 1972). The esterases demonstrated by Holt's method were characterized further using specific inhibitors and activators and following procedures and interpretations given by Pearse (1972) and Hassall and Jennings (1975).

Negative controls for the enzyme studies consisted of heat inactivated specimens and sections (held at 90° C for two minutes before incubation) and the omission of specific substrates from incubation media; positive controls consisted of simultaneous processing of appropriate mammalian and molluscan tissues.

The structure of the proboscis was also studied by digesting the head at room temperature in 0.5% pepsin in 0.2% hydrochloric acid; the various components of the jaw apparatus could then be separated by gentle pressure on a coverslip placed over the preparation. The operation of the proboscis was investigated by direct observation of living *H. homari in situ* on excised host gill filaments and epipodite setae. This was supplemented by photographing the proboscis in movement using "Ilford Mark V" 16 mm negative film at sixty-four frames per second in a Paillard Bolex H 16 cine camera with subsequent frame-by-frame analysis in an L. W. 900 B Motion Analyzer (L. W. Photo Inc., Van Nuys, California).

The nature of the food and the site and sequence of its digestion were studied partly by direct observation of living *H. homari,* as described above, but principally from examination of the gut contents of individuals fixed immediately after removal from the host and then subjected to one or other of the various histological and histochemical routines. The host's gill filaments, epipodites, epipodite setae and the lining of its branchial chamber were similarly examined, to ascertain the source and original condition of the gut contents and to allow differentiation between enzymes ingested as components of the food and those secreted by *H. homari.* pH conditions attending digestion were measured by intra-vital staining with 0.01% sea water solutions of bromo-cresol green (pH range 3.8–5.4), bromo-cresol purple (5.2–6.8), bromo-thymol blue (6.0–7.6) and phenol red (6.8–8.4).

The observations on the cellular structure of the proboscis, stomach and intestine, and on the nature of the food, were supplemented by ultrastructural studies. Fixation, dehydration, impregnation in epon and the preparation of sections for examination with the light and electron microscopes followed the procedures described by Jeon (1965), Parke and Manton (1967) and Jennings (1969).

Observations and Results

Structure of the alimentary canal

The alimentary canal in *Histriobdella homari* (Figs. 1A and 1B) consists of a mouth, small buccal cavity, oesophagus, proventriculus, stomach, intestine and

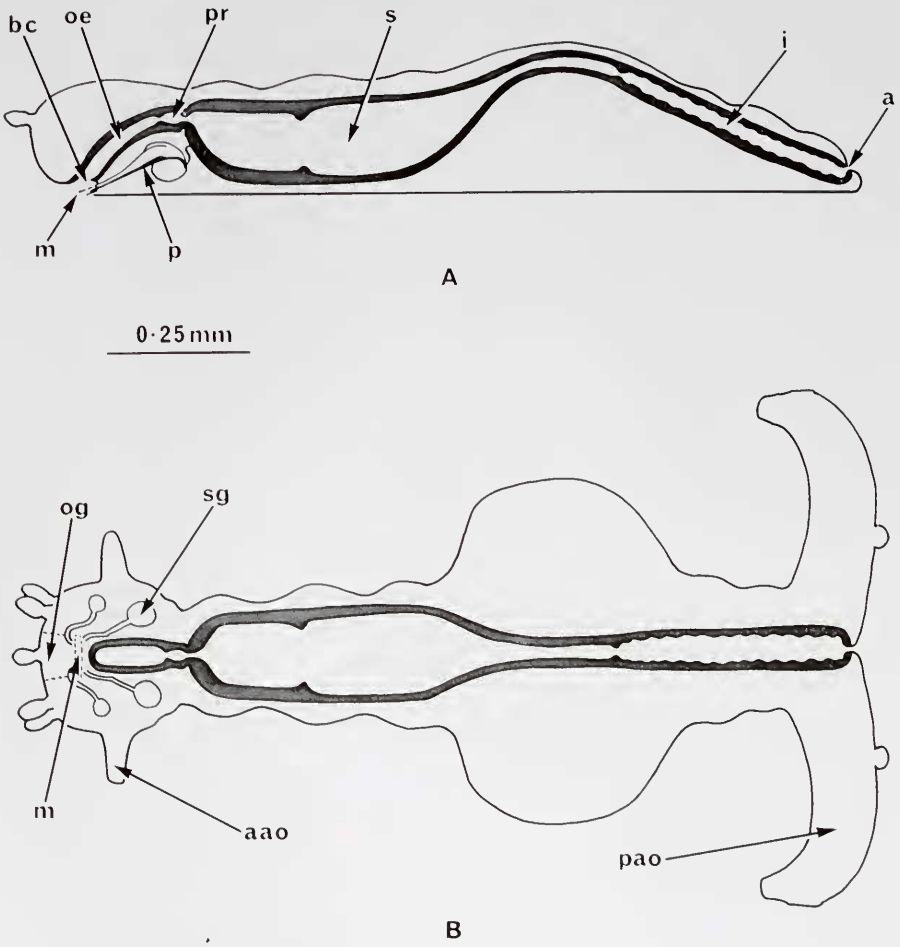

FIGURE 1. *Histriobdella homari*. Schematic longitudinal sections (A, near-sagittal and B, horizontal) through a mature male to show the alimentary canal and proboscis. Abbreviations are: a, anus, aao, anterior adhesive organ; bc, buccal cavity; i, intestine; m, mouth; oe, oesophagus; og, oral groove; p, proboscis; pao, posterior adhesive organ; pr, proventriculus; s, stomach; sg, salivary gland (only two of the nine pairs are shown). The scale bar indicates the overall dimensions; the gut wall is shown thicker than in life.

anus. There is a cluster of unicellular salivary glands on each side of the oesophagus and the modified nonprotrusible proboscis lies in the mid-ventral region of the head below the oesophagus with its anterior portion protruding into the buccal cavity.

The mouth, buccal cavity and proboscis. A shallow oral groove, 30–40 μm wide, originates at the anterior margin of the head and runs posteriorly on the ventral surface to the transverse slit-shaped mouth (Fig. 1B). The mouth is 40–45 μm by 8–10 μm at rest but is capable of considerable distension during feeding. It opens vertically into a small ovoid buccal cavity which in turn leads

dorsally into the oesophagus and receives posteriorly the tips of the maxillae and mandibles of the proboscis (Fig. 1A). The oral groove, mouth and buccal cavity are lined by an unciliated epithelium covered by a thin flexible cuticle, both of which are continuous with those covering the general body surface.

The proboscis (Figs. 1 and 18) is one of the more obvious features of *H. homari,* being easily visible in the light-colored living animal. Previous descriptions have been almost entirely limited to accounts of the sclerotized parts and have varied in detail and accuracy; the most comprehensive account is that by Mesnil and Caullery (1922), but even these authors omit details of the associated musculature and do not attempt the functional interpretation needed for an understanding of the role of the proboscis in the procuring and ingestion of food. To do this it is necessary to give here a full description of the entire proboscis apparatus as observed in the present study. Previous accounts have used different terms to describe the same sclerotized parts, leading to considerable confusion; but, in view of the eunicid nature of the histriobdellids, the basic terminology used by Dales (1962) to describe the proboscis and jaws of typical eunicids will be adopted whenever obvious homologies permit. Synonyms from Haswell (1900, 1913), Shearer (1910) and Mesnil and Caullery (1922) are given in parentheses.

The proboscis in *H. homari* (Figs. 2–14) is 100–120 μm long and 40–45 μm wide. Its hard components are composed of a tough dark sclerotized material and consist of two parallel ventral mandibles, a single flexible median dorsal rod ("fulcrum"), paired series of denticulate maxillae ("rami" and "ramules") and a single transverse carrier ("bridle") which slides backward and forward upon the mandibles. Muscular components include a posterior muscular organ, subdivided into a ventral subspherical bulb and a dorsal pyriform structure which has anterior muscular extensions, lateral paired retractor muscles, posterior dorsoventral muscles which anchor the posterior muscular organ to the oesophagus and walls of the head, and anterior muscles which link the maxillae and mandibles to the two major longitudinal muscle blocks of the general body musculature.

The mandibles (Figs. 2 and 12) are rigid fixed structures 95–110 μm long which lie ventrally behind the buccal cavity and below the oesophagus. Each is expanded anteriorly into an outwardly directed hook-shaped portion and an inner rhomboidal plate which touches, but does not fuse with, its fellow on the other mandible. The anterior margins of the plates and hooks are serrated and protrude through the buccal epithelium into the rear of the buccal cavity; the posterior margins are thickened and heavily sclerotized. Each plate is perforated near its posterior margin by an aperture 1–1.5 μm in diameter; the two apertures are linked by a solid non-elastic strand of hyaline tissue which runs between them across the ventral surfaces of the plates, and this holds the plates together.

Behind these expansions the mandibles become progressively more j-shaped in cross section (Figs. 12C and 12D). They develop a vertical plate on their outer margins 3–4 μm high, whose upper edge is thickened over most of its length into a rod 1.5–2 μm in diameter, and also expand laterally on their inner surfaces into blade-like processes which are shallowly curved in cross-section and almost meet in the mid-line. Posteriorly the mandibles are attached to the flattened dorsal surface of the ventral bulb of the posterior muscular organ, retaining their j-shape as they pass over the anterior margin of the bulb but then quickly losing it as their vertical plates taper away (Fig. 3). The horizontal blades become less


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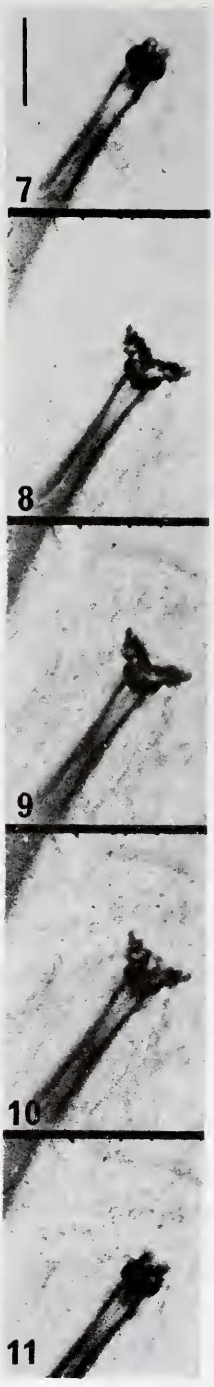
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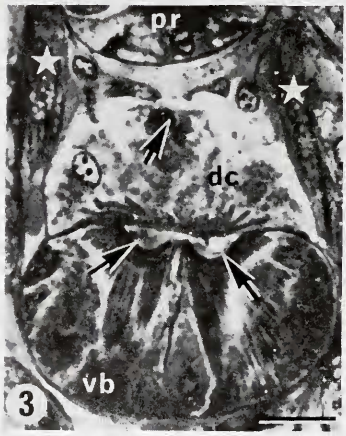
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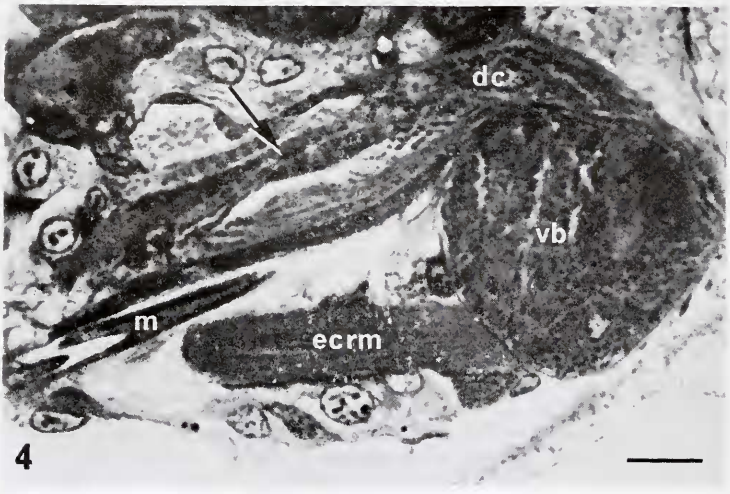
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curved in cross-section and less sclerotized as they pass over the surface of the bulb and curve ventrally around its posterior margin to terminate about one third of the way down the posterior surface (Fig. 14).

The median dorsal rod of the proboscis (Figs. 3, 5, 12, 13 and 14) lies in the mid-line above the mandibles and is inclined from these posteriorly at an angle of approximately 10°. It is 75–80 μm long, 1.5–2 μm in diameter, with a slight dorsal bowing in its posterior half and with its anterior tip slightly bifurcated. The posterior half is embedded within a single narrow elongated cell, 35–40 μm long, which surrounds the rod as a close-fitting sheath and is itself embedded in the upper part of the dorsal component of the posterior muscular organ 12–15 μm above the posterior regions of the mandibles (Figs. 3 and 14).

The posterior end of the rod is surrounded by many folded membranes which anchor it securely within the cell; ultrastructurally these have the appearance of degenerate rough endoplasmic reticulum and thus may well have been concerned with the initial formation of the rod. The cell wall around this region is considerably thickened and consists of a homogeneous matrix enclosed within inner and outer limiting membranes; this thickened area prevents any appreciable backward movement of the rod during operation of the proboscis.

The four pairs of maxillae (Figs 5 and 13) lie above the anterior portions of the mandibles. Each maxilla consists of a series of articulated sclerotized components embedded in the epithelium and musculature of the postero-lateral walls of

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FIGURE 2. *H. homari.* The anterior portions of the mandibles photographed from the dorsal aspect after digestion of the proboscis musculature and removal of other sclerotized components. The right mandible fractured during preparation of the specimen and the longitudinal component is displaced to the right. Scale is 10 μm.

FIGURE 3. Transverse section through the posterior muscular organ of the proboscis. The arrows point to the mandibles and dorsal rod which are seen in cross-section and the stars indicate the dorso-ventral muscles which anchor the proboscis posteriorly; dc, dorsal component; vb, ventral bulb; pr, ventral wall of the proventriculus. The section is of epon-embedded material and is stained with Azur II; scale is 10 μm.

FIGURE 4. Nearly sagittal longitudinal section through the proboscis slightly to the left of the mid-line. The arrow points to the left dorsal rod tensor muscle where this passes over the flexor muscles of the first maxilla; below these can be seen portions of the dorsal rod flexor muscles and the upper margin of the left mandible; dc, dorsal component of the posterior muscular organ; ecrm, left external carrier retractor muscle; m, portion of left mandible; vb, ventral bulb of the posterior muscular organ. The section is of epon-embedded material and is stained with Azur II; scale is 10 μm.

FIGURE 5. The maxillae, carrier and anterior portions of the mandibles and dorsal rod from the dorsal aspect after digestion of the proboscis musculature. The dorsal rod is displaced to the left. The arrow indicates the right-hand expanded wing of the carrier; scale is 10 μm.

FIGURE 6. The carrier from the dorsal aspect after separation from the rest of the proboscis apparatus; scale is 10 μm.

FIGURES 7–11. Five consecutive frames from a filmed record of the proboscis in action, ventral aspect, showing an uninterrupted cycle of proboscis movements without pause for independent operation of the first maxillae. The frames show the carrier and maxillae in the course of protraction (Fig. 7), the point of maximum extension of the maxillae (Fig. 8), stages in retraction of the carrier and maxillae during the effective feeding stroke (Figs. 9 and 10), and the point of maximum retraction (Fig. 11). Photographed at 64 frames per second, giving a time of approximately 75 milliseconds for the complete uninterrupted cycle. Scale is 50 μm.

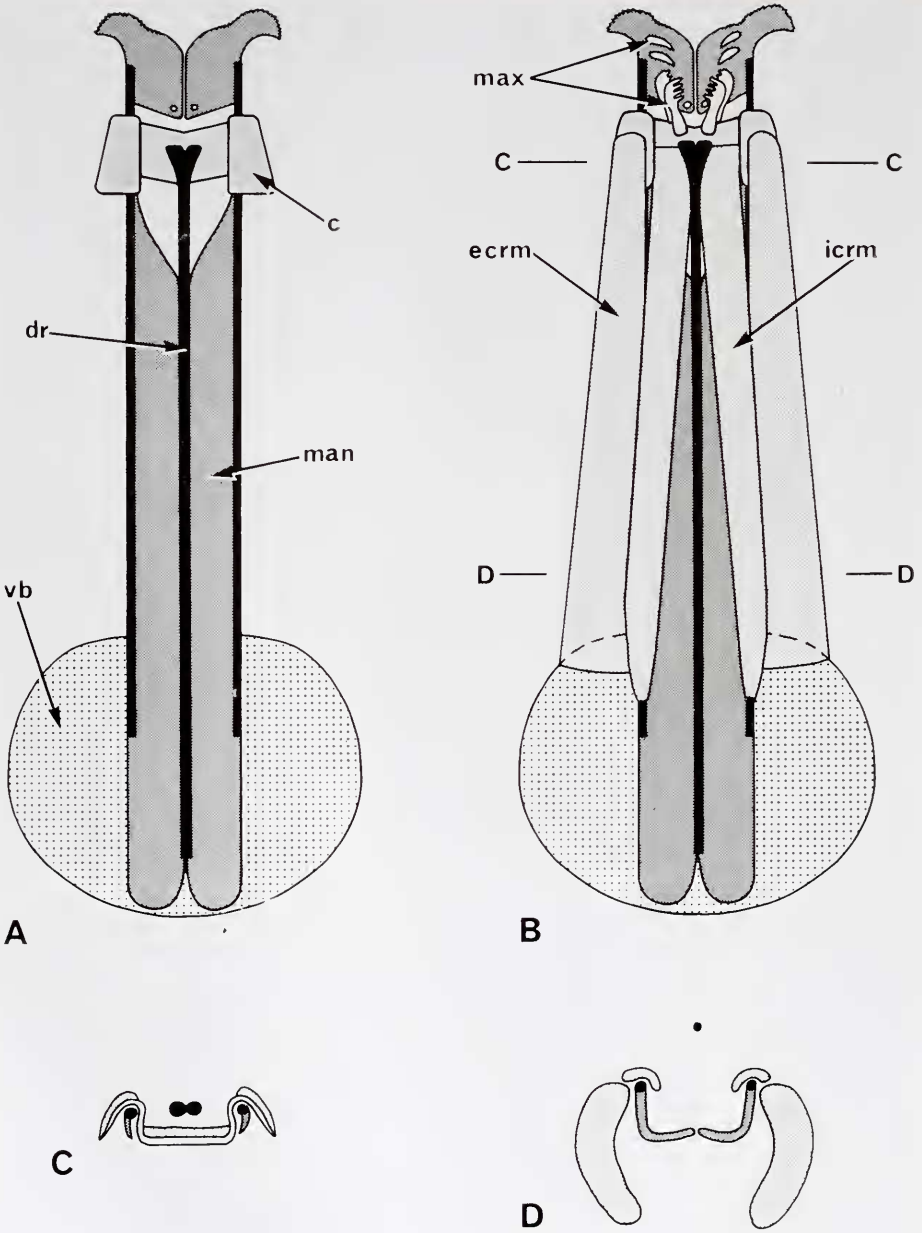


FIGURE 12. *H. homari.* Schematic diagrams of the proboscis to show the arrangement and relationships of the parts, excluding the dorsal component of the posterior muscular organ and the muscles derived from it. A) The mandibles, carrier, dorsal rod and ventral bulb from the dorsal aspect. B) As A, but with the addition of the carrier retractor muscles and the maxillae. C) Transverse section through the proboscis in the plane C-C. D) Transverse section in the plane D-D. Abbreviations are: c, carrier; dr, dorsal rod; ecrm, external carrier retractor muscle; icrm, internal carrier retractor muscle; man, mandible; max, maxillae; vb, ventral bulb of the posterior muscular organ.

the buccal cavity. The number, size and shape of the component pieces varies in the different maxillae, but the distal one is always the largest; it protrudes from the buccal epithelium into the buccal cavity and bears on its exposed surface either teeth or series of ridges.

The innermost pair of maxillae, which will be called the first maxillae, are 12–15 μm long and 4–5 μm wide. They each consist of a small cuboidal basal element, which articulates proximally with the tip of its respective branch of the bifurcated anterior end of the dorsal rod, and a much larger columnar component. The latter articulates at its base with the distal end of the basal element, and most of its length protrudes from the buccal epithelium. The distal third of the inner surface of the exposed portion of the column, *i.e.*, the surface facing the other first maxilla, is produced into four large spike-like teeth. These toothed components, unlike their homologues on the second, third and fourth maxillae, can be moved independently of the other parts of the maxillary apparatus. They can be swung inward, from a position in which they lie almost parallel to the surface of the buccal epithelium, through an arc of 70–80° (Fig. 13A) almost to meet before returning to their original position.

The second maxillae are 15–20 μm long and lie ventrally to the first pair. Each consists of three components: a basal piece which lies below the corresponding element of the first maxilla of its side and, like that, articulates with the dorsal rod, a small median element and a columnar distal component. The latter is larger than its homologue of the first maxilla but is less exposed, having the greater part of its column embedded in the buccal epithelium (Fig. 13). The exposed surface bears eight transverse ridges which give it a file-like appearance.

The third maxillae are 25–30 μm long and are each composed of six rod-shaped elements. The first three elements are arranged linearly, with the proximal one articulating with the basal region of the columnar distal component of the second maxilla. The remaining three elements form a triangle which lies at an acute angle to the line of the first three, with the distal component forming the hypotenuse (Fig. 13). This distal component is 7–8 μm long and 2–3 μm wide; it is slightly curved along its long axis and its exposed surface bears nine transverse ridges.

The fourth maxillae are 12–14 μm long and are each composed of four roughly rod-shaped elements which are arranged in a similar pattern to those of the third maxillae. The proximal element articulates with the third element of the third maxilla and the distal one bears nine transverse ridges on its exposed surface.

The carrier (Figs. 6 and 12) is a trough-shaped structure 4–5 μm long, 8–9 μm wide and 2–3 μm deep which lies between the mandibles anteriorly. Dorsally it has lateral wing-like expansions 3–4 μm wide and 8–9 μm long which rest on the vertical blades of the mandibles so that the carrier is suspended between these (Fig. 12C). During operation of the proboscis apparatus the carrier slides backward and forward along the mandibles over a distance of 12–15 μm from the anterior end of the vertical blades.

A series of fine inelastic fibers ascend inward from the base and walls of the anterior half of the carrier to the two short limbs of the bifurcated tip of the dorsal rod. These bind the rod to the carrier so that movement of the carrier along the mandibles causes a similar movement of the rod tip, and *vice versa*. The link is not rigid, however, and the carrier and rod tip can move independently of each other over 1–2 μm.

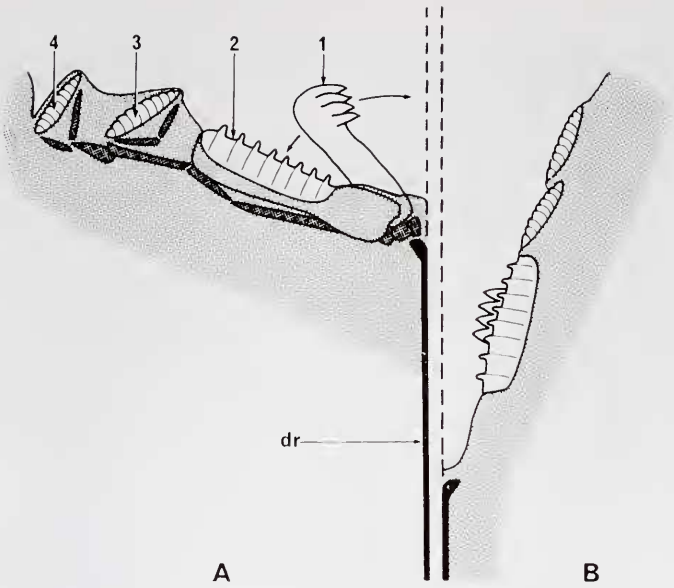


FIGURE 13. *H. homari.* Schematic diagrams of the maxillae from the ventral aspect showing A) the positions of the right maxillae when fully extended laterally and B) the positions of the left maxillae when partially retracted into the resting position. 1, 2, 3 and 4 indicate the exposed denticulate or ridged surfaces of the distal components of the first, second, third and fourth maxillae; the arrows on each side of the first maxilla indicate its arc of movement; dr indicates the right half of the dorsal rod. The dorsal rod and the embedded components of maxillae 1–4 are shown in black.

Similar fibers ascend outward from the anterior margins of the base and sides of the carrier to the basal components of the first and second maxillae, while others run posteriorly from these structures to the two limbs of the dorsal rod.

The musculature of the proboscis is concerned with protraction and retraction of the maxillae relative to the mandibles during feeding, and with anchoring the entire apparatus within the head. The ventral bulb of the posterior muscular organ (Figs. 3, 4, 12 and 14) has the form of a truncated sphere, 25 μm tall and 36 μm in diameter, and is composed of ten conical muscle cells enclosed within a thin membranous sheath. The cells have large basal nuclei with prominent masses of chromatin and contain peripheral bundles of striated fibers (Figs. 3 and 4), very similar to those of the muscle cells of the general body musculature, and many large mitochondria. The bundles of fibers run the length of the cells and converge in the apical regions beneath the ventral surfaces of the mandibles where these are attached to the bulb; their function appears to be the establishment and maintenance of tension within the bulb during operation of the proboscis apparatus and especially during contraction of the external carrier retractor muscles whose posterior ends are attached to each side of the bulb.

The mitochondria are interspersed between the bundles in the peripheral regions of the cells and are densely packed together in the inner regions where they almost obliterate other organelles.

The dorsal component of the posterior muscular organ (Figs. 3 and 4) is a dome-shaped structure, 12–14 μm tall and 25–30 μm wide, which lies above the ventral bulb with its posterior region extending ventrally over the postero-lateral surfaces of the latter for 5–6 μm (Fig. 14A). It has one median, two lateral and two dorso-lateral anterior extensions which form muscles concerned with the functioning of the dorsal rod in the operation of the proboscis apparatus and the movements of the first maxillae.

The dorsal component consists of nine large muscle cells which, like those of the ventral bulb, contain bundles of striated fibers and many mitochondria. They are, however, oriented at right angles to the ventral bulb cells, so that their long axes are parallel to the mandibles and dorsal rod. The nuclei and most of the

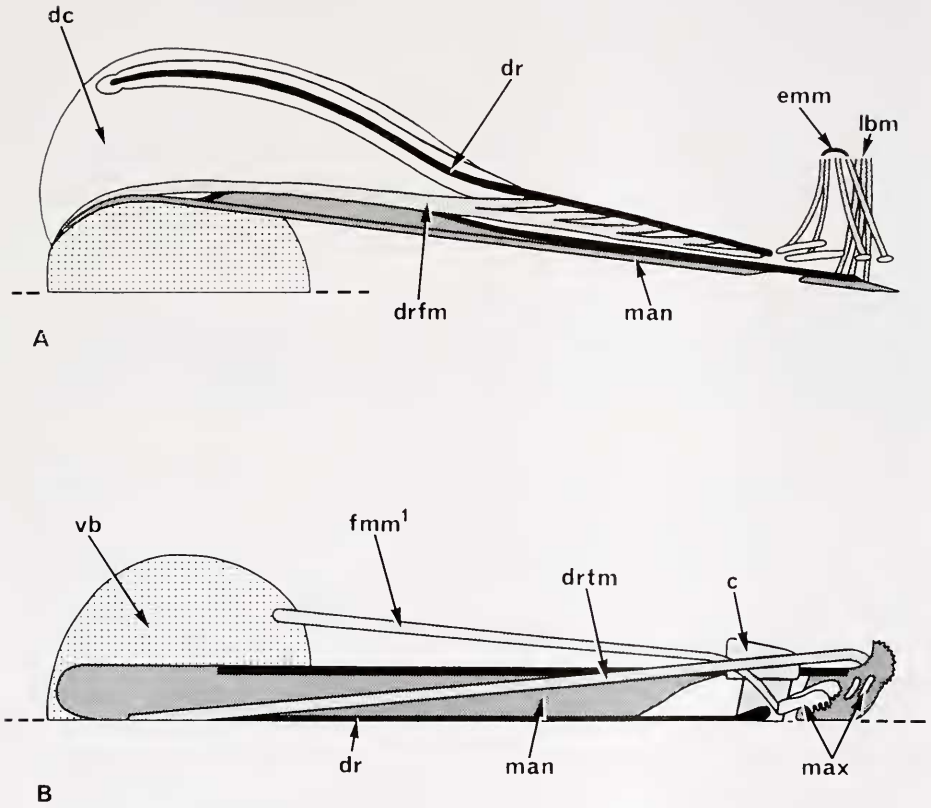


FIGURE 14. *H. homari.* Schematic projections of the dorsal region of the proboscis (left side) to show the distribution of the muscles associated with the dorsal rod and maxillae. A) Sagittal projection of the left half; for clarity the carrier is not represented. B) Dorsal projection; cell bodies of dorsal component not represented. Abbreviations are: c, carrier; dc, dorsal component of the posterior muscular organ; dr, dorsal rod; drfm, dorsal rod flexor muscle; drtm, dorsal rod tensor muscle; emm, extensor muscles of the maxillae; fmm^1, flexor muscle of the first maxilla; lbm, anterior ends of the longitudinal body muscles which are attached to the mandibular hook; man, mandible; max, maxillae; vb, ventral bulb.

mitochondria lie in the broader posterior regions of the cells, which make up the body of the dorsal component; the bundles of striated fibers also originate here and pass forward into the anterior extensions of the cells (Fig. 4).

The nine cells are arranged in two tiers. The upper tier contains only two cells; these lie one on each side of the narrow elongated cell which contains the posterior portion of the dorsal rod. The two cells extend over this inner cell as a thin layer 5–6 μm thick, but the bulk of their bodies lies laterally and ventrally to it. The anterior portion of each cell extends forward and downward, diverging from the midline, and becomes attached to the posterior margin of the mandibular hook of its respective side. Two bundles of striated fibers originate in the regions immediately adjacent to the posterior tip of the dorsal rod and run forward for the full length of the cells to their insertion on the mandibular hooks. The two cells thus form a pair of muscles which help prevent any backward movement of the rod during operation of the proboscis, and are named, therefore, the dorsal rod tensor muscles (Figs. 4 and 14B, drtm).

Five of the seven cells in the lower tier are arranged so that their posterior portions form a compact block, and it is the postero-lateral margins of this which extend downward over the ventral bulb (Fig. 14A). Each cell contains a single bundle of striated fibers which originates in that part of the cell overlapping the ventral bulb. The bundles pass into the anterior extensions of the cells which run forward beneath the dorsal rod and become successively attached to the ventral surface of its anterior half, beginning just beyond the point where the rod arches posteriorly.

The five cells thus form a single functional unit whose contraction supplements the action of the carrier retractor muscles, which will be described later, in causing posterior movement of the rod tip and carrier and concomitant bowing of the dorsal rod. The unit is named, therefore, the dorsal rod flexor muscle (Fig. 14A, drfm).

The remaining two cells of the dorsal component lie antero-laterally to the block of five cells. The anterior extension of each cell, containing a single bundle of striated fibers, runs forward to the first maxilla of its respective side and is attached to the toothed distal component. Contraction of the fibers causes this to swing inward towards its fellow of the other first maxilla; the two cells thus form a pair of muscles responsible for flexure of these structures and are consequently named the flexor muscles of the first maxillae (Fig. 14B, fmm^1).

Two pairs of muscles, the external and internal carrier retractor muscles, run from the ventral bulb of the posterior muscular organ to the carrier (Figs. 4 and 12). The external retractors run along the outer sides of the mandibles; each consists of a single multinucleate cell which is kidney-shaped in cross section over most of its length with the concave surface facing inward (Fig. 12D). The cell is 45–50 μm long and 20–22 μm tall posteriorly where it is attached to the ventral bulb. It tapers anteriorly, while still retaining its characteristic cross-sectional shape, and becomes attached to the dorsal surfaces of the lateral wing-like extensions of the carrier (Figs. 12B and 12C).

The internal carrier retractors are attached posteriorly to the upper part of the vertical blades of the mandibles, where these pass over the anterior margin of the ventral bulb (Fig. 12B). Each consists of a single mononucleate cell which has the same kidney-shaped cross section as the external retractors but they are

oriented so that the concave surface lies over the upper thickened edge of the mandibles (Fig. 12D). They run forward and slightly inward from their point of attachment, descending gradually until they become attached to the floor of the carrier (Fig. 12C).

The four cells forming the external and internal carrier retractor muscles resemble the muscle cells of the posterior muscular organ in that they contain bundles of striated fibers and very many large mitochondria, the only significant difference being the multinucleate condition of the external retractors. These each have four large nuclei in their posterior portions but there are no traces of any internal partitions of the cell.

The bundles of fibers in the external retractor muscle cells are attached posteriorly to the internal surface of the cell membrane; the points of attachment lie directly over the attachments of many of the fibers within the ventral bulb cells, and thus the two sets of fibers form an antagonistic system. Contraction of the bulb fibers causes rigidity of the bulb, as described earlier, and this provides a firm basis for the contraction of the external retractor fibers comparable to that provided by the vertical blade of the mandibles for the internal retractors. Contraction of both pairs of retractors pulls the carrier posteriorly along the mandibles and a consequence of this is a similar posterior movement of the maxillae and the anterior tip of the dorsal rod.

The musculature associated with the anterior end of the proboscis is derived from two large longitudinal muscle bands which run the length of the body and are the principal dorsal constituents of the general body musculature. The majority of the bundles of fibers within these muscles terminate in the paired anterior adhesive organs (Fig. 1B) but the remainder descend steeply on each side of the oesophagus toward the mouth. Small bundles of fibers run to the bases of the exposed distal components of the first, second, third and fourth maxillae, while others run to the lateral margins of the mandibular hooks. Contraction of the fibers running to the maxillae causes the distal components to move outward; the fibers therefore constitute extensor muscles of the maxillae (Fig. 14A, emm).

The fibers running to the mandibular hooks do not cause movement of any part of the proboscis apparatus, and they appear simply to be the means of anchoring the longitudinal muscle bands to fixed points within the head, the mandibles being embedded anteriorly in the buccal epithelium and its underlying tissues.

The proboscis apparatus is anchored within the head posteriorly by lateral dorso-ventral muscles (Fig. 3, stars). These run vertically from the ventral wall of the head on each side of the posterior muscular organ and proventriculus, curve inward over the proventriculus and join dorsally in the midline beneath the epidermis. They are thin sheets 2–3 μm thick and 9–10 μm wide which are firmly attached to the sides of the ventral bulb immediately behind the attachments of the external carrier retractor muscles.

A single thin sheet of noncontractile tissue ascends obliquely backward from the mid-posterior surface of the ventral bulb to the anterior wall of the stomach; the function of this connective is not apparent, but it may contribute to the anchoring of the proboscis apparatus.

The elaborate innervation of the proboscis has been described elsewhere (Gelder and Jennings, 1975). It consists basically of a pair of supraproboscidial ganglia linked directly with the brain by two short stout nerves, longitudinal connectives,

transverse commissures, and paired nerves which serve all the various muscular components described here.

Operation of the proboscis. When the proboscis apparatus is at rest, the carrier retractor muscles are slightly contracted so that the carrier lies approximately one third of the way along the length of its posterior travel, with its anterior margin 4–5 μm from the anterior ends of the vertical blades of the mandibles. The flexible dorsal rod, which is attached at its anterior tip to the carrier, is therefore under some tension as backward movement of its posterior end is prevented by the thickened posterior wall of the cell enclosing it and the restraining action of the dorsal rod tensor muscles. This tension, however, is not enough to cause any bowing of the rod in excess of the intrinsic structural curvature of its posterior half. The dorsal rod flexor muscle is slightly contracted, and this holds the anterior tip of the rod down against the carrier so that any upward movement of either the rod or carrier is prevented.

The first and second maxillae are drawn slightly posteriorly since they are connected by their basal elements to both the carrier and the tip of the dorsal rod. The basal elements are firmly embedded in the wall of the buccal cavity and the pull upon them is therefore transmitted to the third and fourth maxillae which are similarly embedded. Thus, in the resting position, the four maxillae of each side lie one behind the other in lines which subtend angles of approximately 20° to the mid-line (shown for the left maxillae, ventral aspect, in Fig. 13B). The extensor muscles of the maxillae are relaxed, allowing withdrawal of the maxillae to this position.

The cycle of movements of the proboscidial components, from this resting position, is initiated by further contraction of the carrier retractor muscles. This pulls the carrier back along the mandibles for another 10–12 μm to the posterior limit of its travel. The force acting on the dorsal rod is thus greatly increased as its anterior end moves posteriorly with the carrier; this is accommodated by an increase in the dorsal curvature of its posterior half. The dorsal rod tensor and flexor muscles also contract and continue to exert their restraining actions on both ends of the rod. The maxillae are pulled further posteriorly and the denticulated series of the distal components almost meet their fellows of the opposite side when the carrier retractor muscles are at maximum contraction. This movement of the maxillae is accompanied by further relaxation of the extensor muscles of the maxillae and some stretching of the posterior wall of the buccal cavity.

Forward movement of the carrier to the anterior limit of its travel and simultaneous forward and outward movement of the maxillae are achieved primarily by sudden relaxation of the carrier retractor muscles, which allows the dorsal rod to return to its original shape by a rapid forward movement of its anterior end. The dorsal rod flexor muscle permits this by relaxing to an appropriate degree, and the extensor muscles of the maxillae actively contribute to it by contracting. The extensor muscles contribute mainly to the outward movement of the maxillae and further supplementation comes from release of tension in the stretched wall of the buccal cavity. As the maxillae move outward continued contraction of the extensor muscles causes the third and fourth maxillae to swing through an arc of 120° so that the exposed ridged surfaces of their distal components come to lie almost at right angles to those of the first and second maxillae (Fig. 13A).

At this point in the cycle, with the maxillae fully extended, there may be a pause of varying duration during which the first maxillae go through an independent set of movements. In these, contraction of the flexor muscles of the first maxillae causes the denticulate distal components to swing inward towards each other through an arc of 70–80° (Fig. 13A); the return movement outward is effected by contraction of the appropriate extensor fibers.

Return of the maxillae, carrier and dorsal rod to the resting position is effected by slight contraction of the carrier retractor muscles and appropriate actions by the other muscular components of the proboscis apparatus.

An uninterrupted cycle of proboscis movements (Figs. 7–11), without independent operation of the first maxillae, occupies on average 75 milliseconds (calculated from an analysis of movements filmed at 64 frames per second which showed that an average cycle occupied only 5 frames). This rate of approximately 12 cycles per second can be maintained for up to 5 seconds when the animal is feeding actively but more usually 2–3 seconds of activity are followed by resting periods varying in duration from a few seconds to many minutes. The pauses within a single cycle, with the maxillae fully extended, may last for 2–3 seconds during which the first maxillae perform their own independent movements at a rate of 10–11 per second.

The salivary glands. Nine pairs of unicellular salivary glands lie in the posterior half of the head (Fig. 15). The gland cells, labelled 1–9 in the Figure, are spherical to oval, 15–17 μm in diameter and produced anteriorly into long narrow ducts which open into the buccal cavity near the maxillae or into the oral groove. They fall into four groups as regards the staining reactions, point of discharge and role of their secretions.

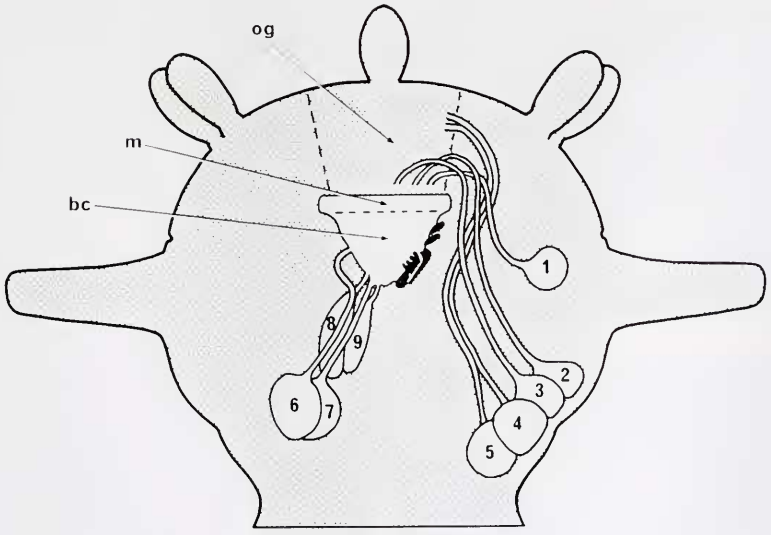
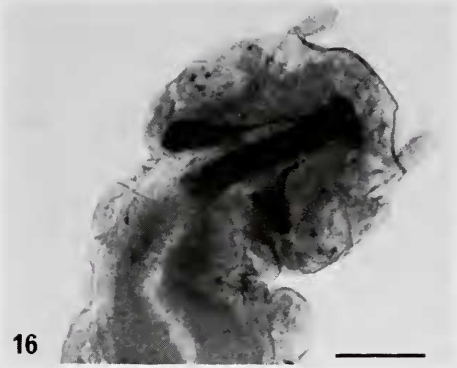


FIGURE 15. *H. homari.* Outline of the head from the dorsal aspect to illustrate the distribution of the salivary glands and their ducts. For clarity only one member of each pair of glands (labelled 1–9) is shown; bc, buccal cavity; m, mouth; og, position of oral groove on ventral surface of head.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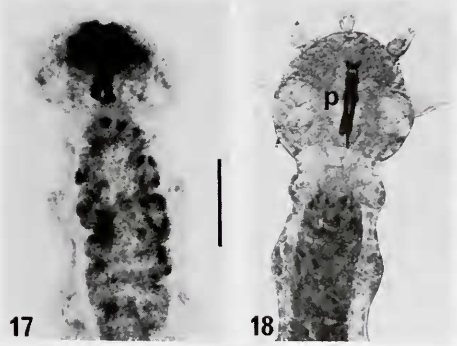
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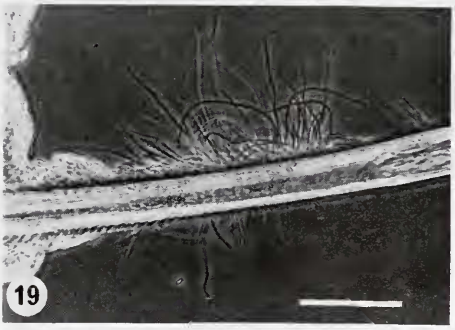
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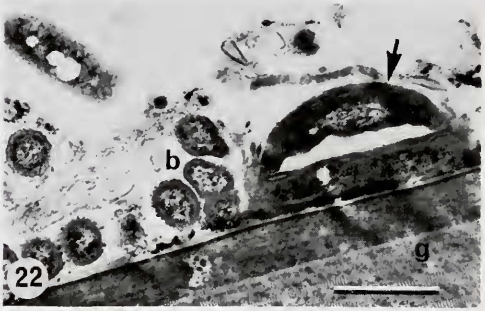
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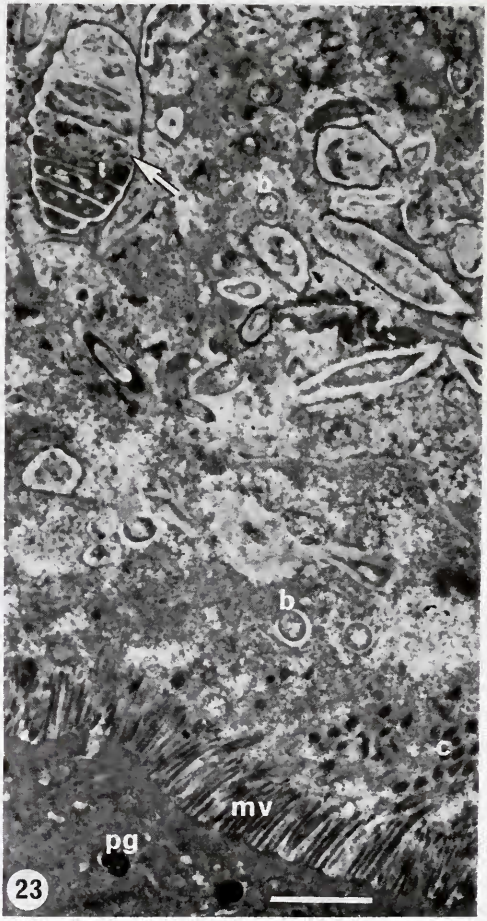
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The secretions of cell pairs 1–5 stain weakly with Alcian blue and periodic acid-Schiff. They are discharged into the oral groove in front of the anterior lip and form the transport medium in which food particles are conveyed into the mouth. Secretions from cell pair 6, in contrast, stain very strongly with Alcian blue (Fig. 16) and periodic acid-Schiff, indicating a high acid mucopolysaccharide content which is probably mucin. They are discharged onto the exposed ridged surfaces of the second and third maxillae and bind together food particles as these are removed from the substratum.

The secretions of cell pair 7 show no reaction to Alcian blue but do stain strongly with periodic acid-Schiff, showing that they have a high carbohydrate content. They are discharged at the junction of the oesophagus and buccal cavity and join incoming food particles as these are drawn upward into the oesophagus.

Cell pairs 8 and 9 produce secretions negative to Alcian blue and periodic acid-Schiff but which give strong positive reactions for C-type esterases. These are identical with the reactions shown by the major component of the secretions produced by the gland cells of the stomach, which will be described later. The salivary esterases are discharged into the buccal cavity below the first maxillae

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FIGURE 16. *H. homari*. The head and anterior body showing the sixth pair of salivary glands, flanking the proboscis, which produce the strongly Alcian blue positive mucous secretions. The whole mount preparation is stained with Alcian blue; scale is 50 μm.

FIGURE 17. Whole mount of *H. homari* showing concentrations of esterases in the gland cells of the stomach, with smaller amounts in the absorptive cells. The dark area in the head surrounding the anterior portion of the proboscis results from esterase activity around the brain and in the eighth and ninth pairs of salivary glands. The whole mount preparation was treated by Holt's method for nonspecific esterases; scale is 100 μm.

FIGURE 18. Whole mount of *H. homari* showing β-glucuronidase activity in the stomach wall. This specimen also demonstrates the prominence of the proboscis (p) as a feature of the head; during fixation the dorsal rod has been displaced posteriorly. The specimen was treated by Pearse's modification of the post-coupling method for β-glucuronidase; scale as in Figure 17.

FIGURE 19. Middle portion of an epipodite seta from *Homarus americanus* photographed by phase-contrast illumination and showing clusters of blue-green algae (center) and the edge of a mass of mucilage-secreting bacteria (left). Scale is 35 μm.

FIGURE 20. Distal portion of an epipodite seta from *Homarus vulgaris* showing a characteristically shaped mucilaginous mass containing numerous bacteria. Photographed by dark-ground illumination; scale is 75 μm.

FIGURE 21. Median portion of a seta from *Homarus vulgaris* showing fringing growths of colorless blue-green algae. The preparation is stained with Alcian blue; scale is 50 μm.

FIGURE 22. Electron micrograph of a section through a gill filament (g) from *H. americanus* showing, on the surface of the filament, profiles of bacteria (b), embedded in a mucilaginous matrix, and of two cells of a blue-green alga (arrowed). Fixation was in glutaraldehyde and osmium tetroxide (following Parke and Manton, 1967); the section is stained with uranyl acetate and lead citrate. Scale is 1 μm.

FIGURE 23. Electron micrograph of a section of *H. homari* fixed immediately after removal from *Homarus americanus*. The section shows the distal region of an absorptive cell in the stomach wall (bottom left) and a characteristic array of ingested microorganisms, contained within a granular matrix, in the stomach lumen. These include part of a filament of blue-green algal cells (arrowed) and bacteria (b). The surface of the absorptive cell bears cilia (c) and microvilli (mv), and two pigment granules (pg) can be seen within the cell. Preparation and staining of the section were the same as for Figure 22; scale is 2 μm.

and are poured onto incoming food particles before these are swept toward the oesophagus.

The oesophagus, proventriculus, stomach and intestine. The alimentary canal beyond the buccal cavity is an unbranched tube whose wall consists of an inner epithelium surrounded by thin layers of inner circular and outer longitudinal muscles. A detailed account of its ultrastructure will be given elsewhere; the present description is limited to basic features necessary for an understanding of the general pattern of digestive physiology.

The oesophagus ascends obliquely backward from the roof of the buccal cavity over the proboscis and joins the proventriculus in the posterior head region (Figs. 1A and 1B). The junction of oesophagus and proventriculus is marked by an internal constriction caused by an increase in the thickness of the lining epithelium; both the oesophagus and proventriculus are densely ciliated and the thickening of the epithelium at their junction results in an effective valvular arrangement of cilia which controls entry of material into the proventriculus.

The proventriculus is a relatively small chamber separated from the stomach by a muscular constriction at the point where the gut passes from the head into the first body segment. The muscular constriction results from thickening of the circular muscle of the gut wall at this point and controls entry of food into the stomach. The epithelium lining the proventriculus is similar to that of the oesophagus, consisting of ciliated cuboidal cells 8–10 μm tall and lacking any glandular components.

The stomach is the largest portion of the alimentary canal (Figs. 1A and 1B) and lies in body segments 1–5. It is expanded over most of its length into a voluminous chamber, almost filling segments 1–3, and tapers posteriorly as it passes over or between the gonads in the male and female, respectively. The anterior third of the chamber has thicker walls than the rest of the stomach, and its posterior limit is marked internally by a ring of very tall columnar cells. There is not, however, any modification of the musculature to form a sphincter and the ridge of cells appears to serve simply as a mechanical partial barrier, enhanced by the cilia of its cells, which contributes to retention of food in this anterior portion of the stomach.

The epithelial lining of the stomach is differentiated into glandular and absorptive cells which are deeply interdigitated with each other. The gland cells occur mainly in the anterior portion of the stomach, where 25–30% of the cells are of this type, but they also occur in smaller numbers posteriorly. They are conical cells 9–10 μm tall and 7–8 μm wide basally, with prominent nuclei. Their apices lack cilia but bear many short tightly packed microvilli. Ultrastructurally the cells are typical secretory structures, with the cisternae of the rough endoplasmic reticulum distended by large amounts of amorphous material. Histochemical techniques reveal that the cells produce organophosphate- and eserine-resistant esterases which are optimally demonstrated in the standard indoxyl acetate incubation medium at pH 4.5 (Fig. 17). The reaction is enhanced by inclusion in the medium of 10^{-3} M cysteine or 10^{-4} M sodium *p*-chloromercuribenzoate, and is 80–90% inhibited by inclusion of 10^{-2} M β-phenylpropionic acid (βPPA). This combination of properties indicates that a mixture of A- and C-esterases is present (Pearse, 1972) with the C-esterases (inhibited by βPPA) predominant. The esterases of the salivary secretions differ from these gastric esterases only in being

totally inhibited by βPPA and appear, therefore, to consist exclusively of C-esterases.

The absorptive cells are cuboidal to trapezoidal, 9–10 μm tall, with large basal nuclei and prominent nucleoli. Their free distal surfaces are uniformly ciliated and bear regular rows of microvilli, 0.3–0.4 μm in length, between the cilia. They contain variable numbers of refractile brown to black pigment granules, 0.5–0.8 μm in diameter, which are insoluble in organic solvents and dilute mineral acids (Figs. 23 and 24). The cells also contain lipid globules which are of the same size range as the pigment granules and, like these, vary in number in different cells. There is, however, little apparent correlation between the numbers of pigment granules and lipid globules persent in any one cell.

Ultrastructurally the absorptive cells differ from the gland cells in that the cisternae of their rough endoplasmic reticulum are much narrower, with parallel walls and less prominent contents. A further difference is the presence in the cells of variable numbers of oval to spherical vesicles 0.4–0.8 μm in diameter which are filled with finely granular material. The pigment granules either occur within these vesicles (Fig. 24) or show remnants of them around their periphery. Small dense bodies, 0.1–0.3 μm in diameter and originating in the Golgi, also occur in the cells and may be found around the vesicles. Histochemical methods show that the absorptive cells produce A- and C-esterases, in smaller amounts than the gland cells but with C-esterases still predominating, β-glucuronidase (Fig. 18) and acid phosphatase. These enzymes were optimally visualized at pH 5.0 and, at the light microscope level, are localized in granules of approximately the same size and distribution as the dense bodies produced by the Golgi. It is concluded, therefore, that the bodies are lysosomes, although they consistently failed to give any reaction for arylamidases which are usual lysosomal constituents.

The only other enzyme demonstrated in the absorptive cells was alkaline phosphatase, which occurs in a narrow distal band 0.5–1.0 μm deep in the cytoplasm immediately below the ciliated distal surface. This zone also contains many mitochondria (Fig. 24) and the enzyme is probably, therefore, of mitochondrial origin.

The junction between the stomach and the intestine in segment 5 is marked by a ring of columnar cells similar to those at the junction of the anterior and posterior chambers of the stomach. The intestine (Figs. 1A and 1B) is narrower than the stomach and runs as a straight tube through segments 6, 7, and 8. It terminates at the anus in the mid-dorsal line on segment 9, between the two posterior adhesive organs formed by the bifurcation of this segment. Its epithelial lining varies in thickness from 4 μm to 8 μm, so that the luminal surface has a slightly corrugated appearance, and the constituent cells closely resemble the absorptive cells of the stomach. They are uniformly ciliated, with regular short microvilli between the cilia, and contain variable quantities of lipid globules and pigment granules. The latter, as in the absorptive cells, are generally enclosed within vesicles or the remnants of vesicles; lysosomes are associated with the vesicles and also occur throughout the cells. The rough endoplasmic reticulum is of the same type as that of the absorptive cells and mitochondria are common immediately below the ciliated distal surface. Only A- and C-esterases and acid phosphatase could be visualized histochemically in the lysosomes; no other enzymes could be demonstrated in the

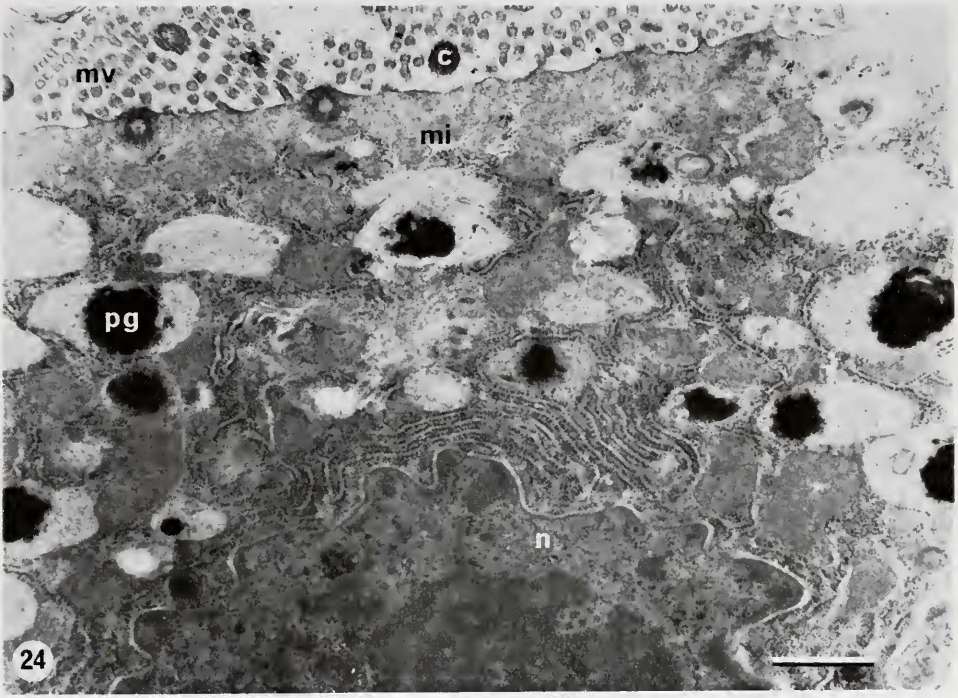

FIGURE 24. *H. homari.* Electron micrograph of a section through the distal half of an absorptive cell from the stomach wall. Abbreviations are: c, cilium; mi, mitochondria; mv, microvilli; n, nucleus; pg, pigment granule within a vesicle and surrounded by finely granular material; preparation as for Figure 22; scale is 1.5 μm.

intestinal cells apart from alkaline phosphatase which occurs in the distal regions of the cells surrounding the anus.

The circular muscle of the gut musculature is thickened around the anal opening to form a distinct sphincter, comparable to that surrounding the junction between the proventriculus and stomach.

The food and feeding mechanism

Forty lobsters were examined during this study (20 *Homarus americanus* in July and August 1973 and 1974, and 20 *H. vulgaris* from April to June and September to December 1974). All carried *H. homari* within the branchial chamber, the numbers present ranging from 5 in a soft-shelled, recently molted *H. americanus* to over 100 in several inter-molt specimens of both species. Every lobster, except for the soft-shelled specimen, also carried rich growths of micro-organisms on the inner surfaces of the branchial chamber, the setae fringing the edges of the carapace, the gill filaments and, especially, the surfaces and setae of the epipodite plates which lie between the gills.

The microfauna included many species of stalked and sessile ciliated protozoa, occasional small colonies of calyptoblastic hydroids such as *Clytia* sp., sessile and

creeping species of rotifers and various adult and larval copepods. The microflora (Figs. 19–22) consisted principally of bacteria and blue-green algae, but some filamentous and spherical unicellular green algae were also present. The bacteria included unbranched chains of large Gram-negative rods, some of which were tentatively identified as members of the Flexibacteriales, and others, including both Gram-positive and Gram-negative rods, which were embedded in mucilaginous masses borne on various structures within the branchial cavity (Figs. 19, 20 and 22). The blue-green algae were generally growing in characteristic rosette-shaped groups of filaments (Figs. 19); they showed the usual blue-green coloration but colorless forms were also present either in rosettes or, more usually, as fringing filaments clothing many of the epipodite setae (Fig. 21).

Examination of the gut contents of over 100 *H. homari* showed that the polychaete feeds exclusively upon the microflora of its habitat, and no traces of materials referable either to the host or the microfauna were found in any part of the alimentary canal. Apart from this major discrimination between substances of plant and animal origin, however, *H. homari* appears to be a relatively unselective feeder and the proportions of bacteria, blue-green and green algae in the gut contents were much the same as in the habitat.

When the polychaete is about to feed, it establishes a secure hold upon the substratum with its posterior and anterior adhesive organs. At this stage the proboscis apparatus is in the resting position (Figs. 11 and 13B). The oral groove and mouth are placed over the food, the anterior lip is drawn forward and upward, and the posterior lip backward and downward. A slight forward movement of the head brings the exposed serrated edges of the mandibles into contact with the food, usually where it is attached to the substratum. The cycle of proboscidial movements, as described earlier, then begins. The maxillae move first posteriorly and then antero-laterally into their fully expanded positions (Figs. 7–10 and 13A). They are then pulled ventrally and inward toward the mid-line (Fig. 11) to complete the cycle. During this ventro-medial movement the toothed surfaces of the first maxillae and the ridged surfaces of the second, third and fourth maxillae draw the food, rake-like, across the serrated margins of the mandibles and detach it from its substratum.

Alternatively, the second, third and fourth maxillae may remain fully extended laterally, for a time, while the first maxillae operate independently, rapidly opposing their toothed surfaces and detaching the longer filamentous food organisms.

The secretions of the nine pairs of salivary glands (Fig. 15) are discharged in sequence during detachment and ingestion of the food. Secretions from the first five pairs of glands, which contain only a small proportion of mucus judging from their staining reactions, are the first to be discharged. They are poured from the roof of the oral groove onto the food as the maxillae commence their movements and are drawn through the buccal cavity into the oesophagus by the action of the oesophageal cilia which are particularly long and densely packed at the junction of the oesophagus and buccal cavity. The secretions from the sixth pair of glands, containing a much larger proportion of mucus, are released as the maxillae detach food particles. They cause the detached particles to adhere together in irregular clumps which are then swept with the secretions from the first five pairs of glands into the oesophagus. As the particles pass through the buccal cavity they receive the esterase-rich secretions from the eighth and ninth

pairs of salivary glands, and, at the point of entry to the oesophagus, they receive the periodic acid-Schiff positive secretions from the seventh pair.

The clumps of food particles and the various salivary secretions are swept up the oesophagus into the proventriculus where they remain until this chamber is full, onward passage into the stomach being prevented by contraction of the sphincter at the proventricular-stomach junction, while the valvular arrangement of the cilia at the proventricular-oesophageal junction prevents return down the oesophagus. During their stay in the proventriculus, which may occupy 1–30 seconds depending on the rate of feeding, the food organisms and salivary secretions are rotated and thoroughly mixed by ciliary action. Eventually the sphincter relaxes briefly, and the contents of the proventriculus are immediately swept into the stomach.

Digestion

Materials entering the stomach consistently show small amounts of A- and C-esterase activity caused by the intrinsic enzymes of the food organisms and the C-esterases contributed by the salivary secretions. The level of esterase activity, however, rises rapidly as the gland cells of the stomach discharge their secretions, which consist mainly of C-esterases. The stomach contents are kept in continuous movement by the cilia of the absorptive cells; this results initially in the efficient mixing of the fluid and solid components during which there is presumably a considerable amount of digestion since many of the ingested microorganisms lose their identity. Bacteria progressively disappear, blue-green algae lose their cellular contents and the granular matrix in which the food organisms are ingested, formed from the mucous salivary secretions and the masses of mucilage surrounding some of the bacteria, becomes more homogeneous (Fig. 23).

The continuous rotation of the stomach contents eventually causes aggregation of solid particles, some of which are still undergoing digestion, into a number of strings. This occurs in the anterior stomach and is particularly noticeable when the ingested food contains a large proportion of mucilaginous bacterial colonies. The strings gradually accumulate in the posterior stomach where they coalesce into oval pellets, 50–100 μm long and 10–20 μm wide. Formation of the strings and pellets effectively separates the solid from the fluid contents of the stomach; the separation is then rapidly followed by progressive decrease in the volume of the fluid component. This is caused by absorption of some of the fluid by the absorptive cells and the passage of the remainder into the intestine where it too is absorbed. Disappearance of fluid from the stomach and intestinal lumina is accompanied by development of vesicles in the epithelial cells (Fig. 24); the contents of these vesicles have the same appearance and staining reactions (faintly acidophilic and Alcian blue and periodic acid-Schiff positive) as the fluid materials in the gut lumen and presumably consist of the digested and semidigested products of the extracellular digestion effected by the esterases from the salivary glands and stomach gland cells. The method of uptake by the epithelial cells is unknown, as no evidence of phagocytosis or pinocytosis was found. It is concluded that materials enter by some type of absorptive process and then collect to form the intracellular vesicles; the abundance of mitochondria in the distal

regions of the cells suggests that this is probably an active, energy-dependent process.

The association with the vesicles of lysosomes containing A- and C-esterases, acid phosphatase and, in the stomach cells, β-glucuronidase, and the occurrence of pigment granules in the vesicles indicates that digestion is completed within these and that the pigment granules are accumulated insoluble end-products.

The intracellular digestion occurring in the intestinal epithelium differs somewhat from that in the stomach absorptive cells in that β-glucuronidase is not involved. Further, the A- and C-esterases demonstrated in the intestinal lysosomes were optimally visualized at pH 7.0–7.5, as compared with pH 5.0 for those in the stomach cells. There must thus be differential absorption in the stomach and intestine of substances whose digestion requires, in its later stages, different enzymes and different pH optima.

Intra-vital staining of *H. homari* with various indicators showed that the pH of the food drops sharply to between pH 4.0 and 5.0 as it enters the stomach, and it remains at this level throughout its stay in this organ. The absorptive cells showed a similar pH value, in those instances where staining of the cells was adequate, and these observations support the histochemical indications that both extra- and intracellular phases of digestion in the stomach occur in an acidic medium. The intestinal cells showed a pH value of between 7.0 and 8.0 and, again, this agrees with the histochemical findings that intracellular digestion in the intestine proceeds in a slightly alkaline medium.

The pellets of indigestible residues eventually pass into the intestine and are then rapidly swept to the anus and expelled. The intracellular residues, however, do not appear to be voided into the gut lumen and are believed to remain in the stomach and intestinal cells throughout life. This conclusion is supported by the absence of pigment granules from the gut of newly hatched *H. homari* and observation of increasing amounts in immature through sexually mature adults.

Movement of food through the alimentary canal is effected primarily by ciliary action; the gut musculature is not especially well developed and does not cause any significant peristaltic movements of the food.

Food reserves

Lipid forms the principal food reserve of *H. homari*. Large quantities occur in the absorptive cells of the stomach and in the intestinal epithelium as globules 0.5–0.8 μm in diameter. These reserves are rapidly depleted if the polychaete is kept away from its host in filtered sea water and disappear after 3–4 days. Their depletion is quickly followed by death, and no isolated individuals survived for longer than five days.

Very small amounts of glycogen occur in the same cells as the lipid reserves and also in the gonads. These disappear within hours of deprivation of food and do not constitute significant long term reserves.

Discussion

These observations show that the relationship between *Histriobdella homari* and its crustacean host has a firm nutritional basis, with the polychaete feeding

exclusively on the microflora of the lobster's branchial chamber. The relationship is thus not detrimental to the host but is, in fact, probably beneficial in that removal of encrusting microorganisms from respiratory structures can only be advantageous. Total removal, of course, effectively occurs when the host molts, but continuous small-scale removal by *H. homari* during inter-molt periods probably prevents excessive build-up of microorganisms. *H. homari,* therefore, can be regarded as an epizoic microphagous cleaning symbiote.

The variety and quantity of the microfloral growths consistently found in the lobster branchial chamber was somewhat surprising. Epizoic blue-green algae have been reported from some other crustacea (Margalef, 1953; Bunting and Lund, 1956; Shelton, 1974) but not in the quantities observed in the present study or in association with other microorganisms; the microflora and fauna of the lobster branchial chamber constitute an interesting and compact ecosystem which merits further study. In the present context, the microflora is seen to provide a rich food source for *H. homari* and its abundance in the specimens examined was probably a major factor contributing to the 100% incidence and high individual host infestation rate of the polychaete. Similar incidence and infestation rates have been recorded, for *Homarus americanus,* by Uzmann (1967) and Simon (1967; 1968) and *H. homari,* therefore, appears to enjoy a considerable degree of success as a symbiote.

The diet, digestive physiology and food reserves of *H. homari* are very similar to those of some other polychaetes which are microfloral grazers (Jennings and Gelder, 1969; Gelder and Uglow, 1973) and appear to be virtually unaffected by adoption of the symbiotic habit. The range of digestive enzymes present is somewhat limited and lacks, for example, exopeptidases which are easily demonstrable, by the arylamidase technique, in carnivorous and sanguinivorous annelids (Jennings and van der Lande, 1967). There is, though, considerable emphasis on production of β-glucuronidase, and both these features are clearly adaptations to the nature of the diet rather than to the mode of life. A comparable situation occurs, for example, in the free-living nematode *Monhystera denticulata* which, like *H. homari,* is microphagous with a high proportion of bacteria in its diet, lacks intestinal exopeptidases, but produces considerable quantities of β-glucuronidase (Jennings and Deutsch, 1975).

The principal adaptive feature in the nutrition of *H. homari,* then, would seem to be in the proboscidial apparatus which is very much modified from the basic eunicid pattern and is more complicated in structure and mode of operation than the feeding mechanisms of most other polychaete microfloral grazers. A possible reason for this is that while the food organisms utilized are of the same type as those taken by free-living grazers their substrata are very different and they are probably more firmly attached to them as an adaptation to the constant flow of water through the host's branchial chamber. Thus, the initial dislodging of the food organisms prior to ingestion may require either greater force or a more specialized reaping-type of mechanism than would be needed to dislodge similar epiphytic or epilithic organisms.

Modification of the eunicid proboscis into the histriobdellid form has involved development of the maxillae into articulated structures, elaboration of the transverse carrier into a vehicle capable of controlled anterior and posterior movement, the use of the dorsal rod (which is probably a modified backward prolongation

of the carrier) to store energy for protraction of the maxillae, and the separation and development of the muscular components. These modifications allow the now-movable maxillae to be used in conjunction with the fixed mandibles as a reaping mechanism which detaches algae and bacteria from their substrata for subsequent ingestion by ciliary action.

The eunicid proboscis appears to have had the potential to evolve in a number of different ways (*vide* Dales, 1962) and the histriobdellid type, as seen in *H homari,* is probably the most elaborate form to have arisen. A comparable modification, but along different lines and resulting in movable scissor-like mandibles, has occurred in the family Ichthyotomidae which contains the single species *Ichthyotomos sanguinarius.* This species, too, is symbiotic but appears to be ectoparasitic rather than epizoic; it lives on eels attached to the gills or fins and uses the mandibles for adhesion and to release blood which is then ingested (Eisig, 1906).

Modification of the proboscis in *H. homari* has been accompanied by specialization of the salivary glands which have become important components of the feeding mechanism, with their secretions performing at least three distinct functions. One of these is to trap microorganisms dislodged by the maxillae and prevent their being swept away by the host's respiratory current, a second is to provide a transport medium for the food and the third is to initiate digestion.

Accounts of other members of the Histriobdellidae by Haswell (1913), Cordero (1927), Lang (1950) and Roubaud (1962), when re-examined in the light of the present findings, indicate that their relationships with their respective hosts are much the same as between *H. homari* and its hosts and that the pattern of nutritional physiology seen in this species is probably characteristic of the entire family. If this is correct, then the Histriobdellidae, as symbiotic polychaetes, are directly comparable to the Temnocephalida which are symbiotic Turbellaria whose general pattern of nutrition is virtually the same as that of their free-living relatives (Jennings, 1971). Both groups live epizoically in the branchial chamber of decapod crustaceans, the histriobdellids as microfloral grazers and the temnocephalids as carnivores. The latter prey, in part, on animals living epizoically in the same habitat and which, therefore, are analogous to the food organisms utilized by the histriobdellids.

A further interesting parallel between the Histriobdellidae and Temnocephalida is seen in their geographical distribution. The temnocephalid-crustacean symbioses occur in fresh water in Australasia, Madagascar and South and Central America; the histriobdellid-crustacean associations have the same distribution apart from the aberrant occurrence of *H. homari* on marine decapods in Northwest Europe and Northeast America. With regard to this last point, it is noteworthy that both temnocephalids and histriobdellids are typical freshwater organisms in that they have eliminated free-swimming ciliated larval stages from their life cycles and hatch as miniature immature adults. This suggests that *H. homari* has at some time become secondarily adapted to the marine habitat, as also must have *Stratiodrilus cirolanae* Führ 1971 which is the only other marine histriobdellid so far recorded.

The geographical distribution of the histriobdellids and temnocephalids indicates a very ancient origin for their associations with crustaceans and, therefore, that the type of relationships in which the modern members of the two groups

are involved probably represent end-points in the evolution of these particular symbioses.

The close parallels in geographical distribution and host types have resulted in some instances in the occurrence of both histriobdellids and temnocephalids on the same individual hosts. *Stratiodrilus tasmanicus* and *Temnocephala quadricornis,* for example, have been found together in the branchial chamber of *Astacopsis tasmanicus* in Tasmania (Haswell, 1900); while *S. platensis* and *T. chilensis* have been recorded from *Aeglea laevis* in Uruguay (Cordero, 1927; Roubaud, 1962). It would be interesting to know whether the *Stratiodrilus* are preyed on by the temnocephalids; if this does occur and the *Stratiodrilus* are feeding on the same type of organisms as *H. homari,* then the branchial chambers of the host crustaceans must indeed contain a complex ecosystem, with components ranging from primary producers to metazoan carnivores.

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Summary

1. The aberrant annelid *Histriobdella homari* (Polychaeta:Eunicida) lives in the branchial chambers of the marine lobsters *Homarus americanus* and *H. vulgaris* where it feeds on the rich microflora of bacteria, blue-green algae and related organisms which grow on the inner surface of the branchial chamber, the setae fringing the edges of the carapace, the gill filaments and, especially, the surfaces and setae of the epipodite plates between the gills. *H. homari,* therefore, is to be regarded as an epizoic microphagous cleaning symbiote of the lobsters.

2. The alimentary canal consists of mouth, buccal cavity, oesophagus, proventriculus, stomach, intestine and anus. The much-modified proboscis lies ventrally below the oesophagus and proventriculus, with its anterior portions protruding into the rear of the buccal cavity.

3. The proboscis consists of two fixed parallel mandibles, a transverse carrier which slides upon the mandibles and to which is attached, posteriorly, a median flexible dorsal rod and, anteriorly, four pairs of movable articulated maxillae, paired external and internal retractor muscles and various tensor, flexor and extensor muscles.

4. Contraction of the retractor muscles withdraws the carrier and maxillae posteriorly, causing bowing of the dorsal rod which is fixed at its posterior end. Relaxation of the muscles allows the rod to straighten and, thus, causes protraction of the carrier and protraction and lateral expansion of the maxillae. Contraction and relaxation of the retractor muscles are supplemented by appropriate changes in the other muscular components of the proboscis.

5. During feeding the serrated anterior ends of the mandibles are applied to the food, the maxillae are fully expanded and then drawn ventro-posteriorly toward the mid-line by contraction of the retractor muscles in the effective movement of the feeding mechanism. This draws the food organisms across the anterior ends of the mandibles, detaching them from the substratum and allowing their ingestion by ciliary action. The first pair of maxillae are also capable of independent action and can be used while the remainder of the proboscis apparatus is held in the protracted position.

6. Detached microorganisms are entangled in a sticky mucous secretion from the salivary glands; other salivary secretions provide a transport medium for the clumped particles and a third set contain C-esterases which initiate digestion.

7. Ingested food is held briefly in the proventriculus, then passed to the stomach where gland cells secrete A- and C-esterases which continue and extend the digestion initiated by the salivary C-esterases.

8. Some soluble products of gastric digestion are taken up by absorptive cells in the stomach wall and their digestion is completed intracellularly by enzymes which include β-glucuronidase. Others pass into the intestine for absorption and completion of digestion by cells similar to the gastric absorptive cells but which lack β-glucuronidase. Insoluble residues of intracellular digestion accumulate in the stomach and intestinal cells as pigmented granules; residues of extracellular digestion aggregate in pellets and are voided through the anus.

9. Lipid forms the principal food reserve and is laid down in the absorptive cells of the stomach and intestine.

10. The histriobdellid-crustacean type of symbiosis, as exemplified by *H. homari* and its lobster hosts, is compared with the temnocephalid (Platyhelminthes: Turbellaria)-crustacean type. Basic similarities in the relative lack of specialization in the nutritional physiology of the annelid and platyhelminth symbiotes, when compared with that of their free-living relatives, are discussed, as also are the implications of known similarities in their life histories and geographical distributions.

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